"Draftsperson's Patent Drawing Review" in this application dated December 10, 1999, by supplying a new formal drawing. Applicants are appreciative of the permission given in the action to defer submission of this formal drawing until allowance of this application.

2. New Grounds of Rejection

By this amendment all new rejections have been carefully reviewed and an earnest effort has been made to amend the claims either to obviate the rejections made, or in one or two instances, to explain the reasons that Applicants believe justify making no changes.

A. Rejection Advanced in Numbered Par. 4, pp. 3-4 of the Action

Parts of this rejection--and specifically, the statement that "There is no support in the specification for the use of any purified antibody" (p. 3 of the Office Action)-- seem to be contradicted by the immediately following sentence "There is only support for using the purified raw polyclonal antibodies" (pp. 3-4 of the Action).

In fact, the specification at p. 5 needs to be read in context with the immediate preceding passage on p.4 which points out that because the ICT assay described and claimed in copending commonly assigned Application Serial No. 09/139,720 exhibited a level of sensitivity of only between 500 and 1,000 colony forming units ("CFU") of bacteria per milliliter of environmental water sampled, the modified enzyme immunoassay disclosed and claimed in this application was developed, using "raw polyclonal antibodies that have first been purified according to the affinity purification procedure described and claimed in the parent

application" (Specification p. 4, lines 12-13), in a modified enzyme assay, to coat test tubes. In the ensuing sentence, the specification mentions that sample and an enzyme conjugate of "the same affinity purified and antigen specific polyclonal antibodies" is added to the coated tube. (p. 4, 14-15).

On page 5 of the specification, the first sentence points out that the use of *the same* "antigen specific antibodies obtained by affinity purifying raw polyclonal antibodies" (p. 5, lines 1-3) is critical to the success of the ICT test for *Legionella pneumophil*a serogroup 1 described and claimed in Serial No. 09/139,720 and the success of the EIA test for *Legionella pneumophila* serogroup 1 described and claimed in this application. The following sentence contrasts these highly successful and useful tests with the analogous unsuccessful tests in which *unpurified* polyclonal antibodies were substituted for the affinity purified, antigen-specific antibodies made by affinity purifying polyclonal antibodies as fully described in patent application Serial No. 09/139,720.

The essential element in both successful tests, however, is *not* the use of "raw polyclonal antibodies "as asserted at p. 3 of the Office Action. The antibodies actually used in them are *not* raw and whether they are still polyclonal *after* affinity purification is an open question which has not been definitively explored. Instead, the essential element of the successful tests is beginning with polyclonal antibodies but using them in the test only after they have been affinity purified, as described fully in parent application Serial No. 09/139,720, to produce antibodies that are antigen specific and affinity *purified.* Under the circumstances, Applicants believe that the term "purified antigen-specific antibodies", used in conjunction with the recitation of the process for obtaining them, is fully supported by the specification and

that it is synonymous with the term "affinity-purified polyclonal antibodies produced by the affinity purification set forth in the parent application" but *not* with the term "purified raw polyclonal antibodies" suggested by the Examiner in the action, which term is open-ended as to the nature of the purification procedure. *Mere* " purified raw polyclonal antibodies" might simply have been conventionally separated, e.g., from the animal serum in which they were obtained from a rabbit or other host animal or they might be an Ig G cut obtained according to a well known technique, neither of which per se, would be successful in the ICT assay of Ser. No. 09/139,720 or the modified enzyme assay of this application. Moreover, minimally "purified" raw polyclonal antibodies are clearly *not* what Applicants' specification is talking about on pages 4-5; indeed the expression "purified raw polyclonal antibodies" is extremely broad if the purification method is not specified, and it would encompass many antibody preparations that could *not* produce the high sensitivity for the characteristic O-carbohydrate antigens of *Legionella* species, and serogroups of species, that is disclosed in this application and its parent application.

The Action has taken out of context the reference on p. 5 of the specification to "unpurified raw polyclonal antibodies" which refers *in context* to polyclonal antibodies that were *not* subjected to the *specific affinity* purification process that results from following steps (a) ,(b) ,(c) and (d) of Claims 10 and 25.

With this explanation and a consideration of pages 4 and 5 of the specification *in context,* it is urged that claims 10 and 25 do not encompass new matter and are not nearly as broad and inclusive of inoperable embodiments as they would be if the term "purified raw polyclonal antibodies" were to be substituted for "purified antigen-specific antibodies" as a

characterization of the product obtained by sequentially following steps (a), (b), (c) and (d) of each of these claims.

B. Rejection of Claim 23 (par. 5 at p. 4 of the Office Action)

Applicant traverses this rejection. The specification at page 6 (lines 15 and 16) in a short separate paragraph states. "In lieu of filtering, the water sample may be centrifuged at high speed and the sample remaining after decanting or aspirating off the water may be transferred to the device".

The swabbing step and delivery of the swab to the test reaction vessel are disclosed in lines 1-5 of the same page and in the "Immunoassay Procedure" on p. 9 at lines 10-13 of that page. See also p. 12, lines, 15-17.

C. Rejection Set Forth in Par. 6, pp. 4-5 of Office Action

By this amendment, the references in the claims to carbohydrate antigens, including those which refer to the essentially protein-free embodiment of the antigen have all been amended to recite an "O-carbohydrate" antigen. Applicant continues to object to "purified raw polyclonal antibodies "as a *claim* term in that it is overly broad. It may also be misdescriptive, because Applicants have not characterized the affinity -purified, originally polyclonal, antibodies as to their chemical nature or the specific determinants they bear. Their unusually high specificity for the characteristic O-carbohydrate antigen of the *Legionella* species, or serogroup of a species, against which they were originally raised poses the question of whether they *are* "polyclonal" or not, after the affinity purification obtained by following steps (a) - (d)

of Claims 1 and 25 has been completed--but Applicants do not know the answer to that. Under the circumstances calling them"purified polyclonal" or "purified raw polyclonal antibodies" *in the claims* rather than as a part of the description of their original condition in an explanatory portion of the specification is unclear and even misleading as well as unduly broad and inclusive of inoperable embodiments.

D. Rejection of Paragraph 7, pp. 5-6 of the Action

The essentially protein-free embodiments of the O-carbohydrate antigens of *Legionella* species, and serogroups of species, differ from the O-carbohydrate antigens of the same species or serogroup of a species in their natural state as a result of the treatment procedure recited in (b) part of Claims 10 and 25 which, at the least, has rendered them essentially protein -free as compared to their naturally occurring counterparts. Applicants have not made a detailed chemical and physical characterization of the essentially protein-free embodiments and likewise have not made such a detailed characterization of the naturally occurring counterparts. To maintain a line of distinction in the claims, Applicants have referred in them as now presented to the "essentially protein-free O-carbohydrate antigen embodiment" as the entity used in affinity purification and to the "O-carbohydrate antigen" as both the target of the assay procedures and the untreated antigen as it occurs naturally in the bacteria.

The sole function known to Applicants of the "essentially protein-free O-carbohydrate antigen embodiment" obtained from a *Legionella* species, or serogroup of a species, is in the affinity purification of polyclonal antibodies raised against the same bacteria from which the embodiment was obtained.

The resultant antibodies are highly specific to the *unpurified* antigen in its natural state,

as the specification of this application and its parent application each teach and also show in various of their respective examples.

E. Rejection of Par. 8 p. 6 of the Action

Step (d) of each of Claims 10 and 25 has now been amended to recite

> "passing polyclonal antibodies to the same Legionella species, or serogroup of a species, as that cultured in step (a) over the chromatographic affinity column of step (c) to produce purified antigen-specific antibodies"

Claims 11 and 26 have commensurately been amended to replace the word "raw" with the word "polyclonal".

It is believed that these amendments obviate this rejection--and that, taken together, they help to alleviate those of the Examiner's concerns that prompted the rejections of par. 4 and 7 of this Action.

F. Rejection of Par. 9, p. 6 of the Action

Claims 10 (e)(ii), 17, 25(f)(ii) and 32 all now refer to the "test reaction vessel " as the vessel in which the test reaction, including incubation and subsequently, development of color, chemiluminescence or bioluminescence, all take place.

G. Rejection of Par. 10, p. 7 of the Action

The buffer solution is recited in Claims 10 (e)(iii) and 25 (f)(iii). Claim 21 has been amended to clarify that the buffer solution is placed in the test reaction vessel and the sample on the swab pad is then immersed in it, and Claim 23 has been similarly amended. The buffer

solution is *not* of a critical composition. While a preferred buffer composition is that recited in Claims 22, 24, and 35 which is disclosed in the examples, many buffers are used in enzymeimmunoassays generally and those of ordinary skill in the art will understand that others could be substituted without departure from the present invention.

Applicants are unaware of the recitation of an extraction solution in Serial No. 09/139,720 and have not found such a reference by inspecting the specification and present claims of that application. Applicants further state that, while the buffer *may* perform a subsidiary function in this assay of helping to open up the bacterial cell walls, so that the O-carbohydrate antigen is accessible to react with antibodies, no such function has been definitively established. Its primary and intended function is as a reaction buffer in the incubation step of the modified enzyme immunoassay described and claimed herein.

It is noted that this application, at page 9, line 13-18 *does* disclose a reagent which has a dual purpose of (1) inducing sample flow in the ICT test of the Example set out at that page and (2) assisting cellwall breakdown. That reagent is not said to have been employed in the examples of the modified enzyme immunoassays that are disclosed and claimed in this application, but it is a reasonable inference that such a reagent could be used if needed.

H. Rejection of Paragraphs 11, 12 & 13, p. 7 of the Action

Claim 23 has been corrected to substitute "to" for--the--. Claim 32 has been corrected

to substitute "(f)(iii)" for--(e)(iii)--

Claims 11 and 26 have been corrected as noted in Section E above.

In claim 20, "a" has been substituted for "the".

Claims 21 and 35 each now reflect that the specific antecedents for "the buffer solution" appeared respectively in Claim 10 (e)(iii) and Claim 25 (f)(iii).

I. Rejection 14, p. 7 of the Action

Claim 25, step (e), has been amended to make clear that the sample is suspected of containing *Legionella* bacteria of the same species or serogroup of a species as cultured in step (a) and that the antigens coated on the particles tend to attract the characteristic O-carbohydrate antigens from those bacteria in the sample. It is believed that this amendment and the added recitation that the sample and the aqueous medium containing antibody coated magnetic particles are first mixed sufficiently conveys the point that by adding the coated particles to the sample and mixing the two, a contact between the sample components and the coated magnetic particles is necessarily achieved.

2. Incorporation By Reference

Applicants respectfully call attention to M.P.E.P. §608.01 (p), August 2001 edition which in part I. A. at page 600-79 states in part as follows:

> An application for a patent when filed may incorporate "essential material" by reference to (1) a U.S. patent, (2) a U.S. patent application publication, or (3) a

pending U.S. application, subject to the conditions set forth below.

> "Essential material" is defined as that which is necessary to (1) describe the claimed invention, (2) provide an enabling disclosure of the claimed invention, or (3) describe the best mode (35 U.S.C. 112). In any application which is to issue as a U.S. patent, essential material may not be incorporated by reference to (1) patents or applications published by foreign countries or a regional patent office, (2) non-patent publications, (3) a U.S. patent or application which itself incorporates "essential material" by reference, or (4) a foreign application.

This passage clearly permits incorporation of "essential material" by reference to a pending U.S. application, so long as that application does not itself incorporate "essential material" by reference--and U.S. Serial No. 09/139,720 does not do so.

It is further noted that the material in §608.01(p) of the M.P.E.P., August 2001 Ed., on the ensuing page 600-80 under I.A.2. "Improper Incorporation" is directed to the impropriety of incorporating by reference "essential material " from foreign patents and foreign applications and from non-patent publications and that in the second column of p.600-80 under this same heading, the M.P.E.P. states:

> "Reliance on a commonly assigned copending application by a different inventor may ordinarily be made for the purpose of completing the disclosure. See *In re Fried,* 329F 2d 323,141 USPQ 27, (CCPA 1964) and *General Electric Co.* v. *Brenner,* 407 F.2d 1258, 159 USPQ 335 (D.C. Cir. 1968).

Applicant's attorney notes that to incorporate "essential material " from Application Serial No. 09/139,720 into this application requires what amounts to complete reproduction, or very close to it, in this present application.

The exercise of attempting to determine whether *any* of the disclosure of application Serial No. 09/139,720 can safely be excluded from this application would be an extremely burdensome one because of the very close relationship between them. It would be

unnecessarily costly to the common assignee of the two applications, a small entity. Moreover, counsel signing this paper availed of the incorporation by reference policy for the purpose of ensuring that anyone reading a patent issuing on this application would necessarily be directed to a careful reading of any patent issuing on Application Serial No. 09/139,720 as well. Attempting to incorporate physically the entirety of Application 09/139,720 into this application would also be burdensome, might result in a somewhat disjointed and difficult-to-interpret final document and would result in one almost unpardonably repetitive vis-a-vis any patent that issues from Application No. 09/139,720.

The two applications are not only copending and commonly assigned, but they have one common inventor and are both currently pending before the same Examiner. Both are working their way to allowability and could quite easily wind up issuing simultaneously.

Section 608.01(p) of the M.P.E.P. in its initial portion on p. 600-79 (August 2001 ed.) points out that the incorporation by reference policy aims to minimize the public's burden"to search for and obtain copies of documents incorporated by reference which may not be readily available." This, of course, explains why the general rule is U.S. patents, patent applications and patent publications may be so incorporated, but foreign patents, foreign applications and nonpatent publications may not.

Applicants hereby respectfully request that the requirement set forth in par.15 of the Action under the heading"Incorporation by Reference" either be withdrawn in view of the above remarks and the overall context of M.P.E.P. 608.01(p) or that it be held in abeyance, at least, until it can be determined whether both applications can be cooperatively brought into condition for allowance and can issue either simultaneously or in a sequence such that this

application issues shortly after Application Ser. No. 09/139,720 does.

3. Double Patenting

Applicants acknowledge that there mat be some overlap in claiming between Application Ser.No. 09/139,720 and this application in that certain broader claims of the earlier application may encompass some of the more specific subject matter claimed herein. Applicants submit however, that if the applications issue simultaneously, or if a terminal disclaimer is presented in this application so that any patent issuing on it expires simultaneously with a patent issuing on Application Ser. No. 09/139,720 such overlap is permitted by law and by practice of the Office as well. Applicants and counsel undertake to make every effort to obtain simultaneous issuance, or alternatively if that is not possible, Applicants and their assignee will supply the proper terminal disclaimer in the later issuing of the two applications.

CONCLUSION

It is believed that the present application is now in condition for allowance-or if not, that it can be rendered so with minor additional amendments. To that end, counsel invites the Examiner to telephone her at her office number given below to discuss any still outstanding issues.

Respectfully submitted,

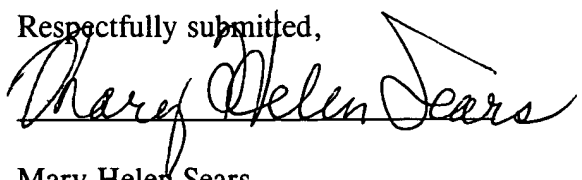

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EXHIBIT A

10 A method [of determining the] sufficiently sensitive to determine a concentration at least as low as from 5 to 50 colony-forming units ("CFU") per milliliter('ml") of water of at least one species, or serogroup of a species, of *Legionell*a bacteria[,] in water suspected of being infected therewith, which method detects an O-carbohydrate antigen characteristic of said at least one species, or serogroup of a species, of *Legionella* bacteria [is sufficiently sensitive to detect said bacteria in concentrations as low as from 5 to 50 colony forming units ("CFU") per milliliter ("ml.") of water] and comprises the following steps:

(a) culturing an identified *Legionella* bacteria species, or serogroup of a species, to a desired size and harvesting therefrom cells of that species, or serogroup of a species, as a wet cell pellet;

(b) obtaining from the wet cell pellet an essentially protein-free embodiment of the characteristic O- carbohydrate antigen of that species, or serogroup of a species, of *Legionella* bacteria carbohydrate antigen by a series of steps which comprises

(i) suspending the wet cell pellet in an alkaline solution and mixing[;] to form a mixture

(ii) adjusting the pH of the mixture to an acid pH with a strong acid;

(iii) separating the mixture from step (ii) into two layers[;], an upper layer and a lower layer;

(iv) removing the upper layer and adjusting its pH to approximate neutrality;

(v) adding to the approximately neutral pH upper layer [product] from step (iv) a broad spectrum protease enzyme and digesting to destroy residual proteins;

(vi) adjusting the pH of the digested product from step (v) to [the] an alkaline [side] pH with a [weakly] dilute aqueous alkaline [aqueous] solution of a weak base and;

(vii) separating out [an] the essentially protein free O-carbohydrate antigen embodiment;

(c) coupling to a chromatographic affinity column through a spacer molecule the essentially protein free-O- carbohydrate antigen embodiment obtained in step (b);

(d) passing polyclonal antibodies to the *same Legionella* species, or serogroup of a species, as that cultured in step (a) over the chromatographic affinity column of step (c) to produce purified antigen-specific antibodies; and

(e) performing an enzyme immunoassay upon a water sample suspected of being infected with *Legionella* bacteria of the same species, or serogroup of a species, as that cultured in step (a), which assay comprises the following steps:

(i) coating a solid substrate with antigen-specific antibodies from

step (d) hereof in an amount sufficient to provide a coating containing at least 0.05 μg per test of said antibodies and allowing the coated substrate to dry;

(ii) preparing a conjugate of an enzyme and purified antigen-specific antibodies from step (d) hereof;

(iii) bringing the sample to be tested and from 0.2 to 2.0 μg conjugate per test into contact with a buffer solution and the coated solid substrate of step (e) (i) in a [suitable] test reaction vessel and incubating for a period of at least 20 minutes; and

(iv) decanting liquid from said vessel, washing, adding a colorimetric, chemiluminescent or bioluminescent material thereto, allowing color, chemiluminescence or bioluminescence to develop for up to about 5 minutes, measuring its intensity in any known manner and determining therefrom the concentration of the suspected *Legionella* bacteria species or serogroup of a species in the original water sample according to predetermined intensity/concentration standards which first correlate the intensity to the amount of O-carbohydrate antigen characteristic of at least said suspected species, or serogroup of a species, of *Legionella* detected in the sample and then correlate that amount in turn to the concentration of the suspected *Legionella* bacteria species, or serogroup of a species in CFU/ml that is present in the water being tested.

11 The method of claim 10 in which the *Legionella* bacteria species, or serogroup of a species, in step (a) is a serogroup of *Legionella pneumophila* bacteria and the [raw] polyclonal antibodies of step (d) are [raw] polyclonal antibodies to bacteria of the same serogroup of *Legionella pneumophila*.

15 A method according to claim 10 [12] wherein the enzyme immunoassay is run as a sandwich assay.

16 A method according to claim 10 [12] wherein the enzyme immunoassay is run as a competitive assay.

17 A method according to claim 10 wherein the coated solid substrate in step (e) (i) is the inner wall of a test tube, which test tube also serves as the test reaction vessel in [step] steps (e) (iii) and (e) (iv).

18 A method according to claim 10 wherein the coated solid substrate of step (e) i is selected from among coated solid inserts and coated beads.

19 A method according to claim 10 wherein the enzyme of the conjugate prepared [reacted]in step (e) (ii) is horseradish peroxidase and a colorimetric agent, tetramethylbenzidine, is added in step (e) (iv).

20 A method according to claim 10 wherein the water to be tested is water obtained from [the] a heating and/or cooling system of a building, or water obtained from a building sanitation or drinking water supply, individual water samples are obtained in quantities of from

100 to 1000 ml. per test and each individual water sample is subjected to a pre-assay concentration step.

21 A method according to claim 20 wherein the concentration step comprises filtering each individual water sample through a filter having a pore size not greater than 0.45μm, and it is followed by collecting filter residue by thoroughly stroking the filter with the swab pad of a swab comprising a handle and an affixed pad of fibrous material or foamed open pore material , and delivering the sample on said swab pad to the test receptacle of step (e) (iii) to which vessel buffer solution and conjugate have already been added, by immersing the swab pad in the buffer solution in said test reaction vessel [contained therein], twirling the pad in said solution and leaving the pad immersed therein throughout the period of incubation set forth in step (e) (iii).

22 A method according to Claim 21 wherein the buffer solution is composed of aqueous 0.05 M tris HCl containing 2-5% of a detergent having a pH of about 7.0.

23 A method according to [the] claim 20 wherein the concentration step comprises subjecting each individual water sample to high speed centrifugation followed by settling and removal by decantation or aspiration of supernatant water and it is followed by thoroughly stroking the residual solids with the swab pad of a swab comprising a handle and an affixed pad of fibrous material or foamed open pore material and delivering the sample on said swab pad to the test reaction vessel [receptacle] of step (e) (iii) to which vessel buffer solution and conjugate have already been added, by immersing the swab pad in the buffer solution [contained therein] and leaving the pad immersed therein throughout the incubation period set forth in step (e) (iii).

24 A method according to claim 23 wherein the buffer solution is composed of aqueous 0.05 M tris [H Cl] HCl containing 2-5% of a detergent having a pH of about 7.0.

25 A method for determining the concentration of at least one species, or serogroup of a species, of *Legionella* bacteria in environmental water suspected of being infected therewith, which method comprises the following steps:

(a) culturing an identified *Legionella* bacteria species, or serogroup of a species, to a desired size and harvesting therefrom cells of that species, or serogroup of a species, as a wet cell pellet;

(b) obtaining from the wet cell pellet an essentially protein-free embodiment of the characteristic O- carbohydrate antigen of that species, or serogroup of a species, of *Legionella* bacteria by a series of steps which comprises

(i) suspending the wet cell pellet in an alkaline solution and mixing to form a mixture;

(ii) adjusting the pH of the mixture to an acid pH with a strong acid;

(iii) separating the mixture from step (ii) into two layers, an upper layer and a lower layer;

(iv) removing the upper layer and adjusting its pH to approximate neutrality;

(v) adding to the approximately neutral pH upper layer[product] from step (iv) a broad spectrum protease enzyme and digesting to destroy residual proteins;

(vi) adjusting the pH of the digested product from step (v) to an [the]

alkaline pH [side] with a dilute [weakly][alkaline]aqueous alkaline solution of a weak base and;

(vii). separating out an essentially protein free O-carbohydrate antigen embodiment;

(c) coupling to a chromatographic affinity column through a spacer molecule the essentially protein-free O-carbohydrate antigen embodiment obtained in step (b);

(d) passing polyclonal antibodies to the *same Legionella* species, or serogroup of a species, as that cultured in step (a) over the chromatographic affinity column of step (c) to produce purified antigen-specific antibodies; [and]

(e) preconcentrating an environmental water[the] sample suspected of containing *Legionella* bacteria of the same species, or serogroup of a species, as that cultured in step (a) by (1) adding thereto and mixing therewith an aqueous medium containing finely divided magnetizable particles which have been precoated with purified antibodies from step (d) hereof, which antibodies tend to draw to themselves characteristic O- carbohydrate antigens of the same *Legionella* bacteria species or serogroup of a species as that cultured in step (a) from the bacteria in the sample and to react therewith to form conjugates, (2) subjecting the mixture of sample and magnetizable particles to the action of a local magnetic field, whereby they are caused to form a coherent mass, (3) decanting or aspirating off the water from the coherent mass and (4) subjecting the mass, in a known manner, to demagnetization and then to elution of the antigen-antibody conjugates from the particles; and

(f) performing an enzyme immunoassay upon the resulting eluate according to the following steps:

(i) coating a solid substrate with antigen-specific antibodies from step (d) hereof in an amount sufficient to provide a coating containing at least 0.05 μg per test of said antibodies and allowing the coated substrate to dry;

(ii) preparing a conjugate of an enzyme with the antibody-antigen conjugates in the eluate from step (e);

(iii) bringing enzyme-antibody-antigen conjugate containing from 0.2 to 2.0 μg per test of enzyme -antibody content into contact with a buffer solution and the coated solid substrate of step (i) in a [suitable] test reaction vessel and incubating for a period of at least twenty minutes; and

(iv) decanting liquid from said vessel, washing, adding a colorimetric, chemilumescent or bioluminescent material thereto, allowing color, chemiluminescence or bioluminescence to develop for up to about 5 minutes, measuring its intensity in any known manner and determining therefrom the concentration of the suspected *Legionella* species, or serogroup of a species, in the original water sample according to predetermined intensity/concentration standards which first correlate the measured intensity to the amount of O-carbohydrate antigen characteristic of at least said suspected species, or serogroup of a

species, of *Legionella* bacteria present in the sample and then correlate that amount in turn to the concentration of the suspected *Legionella* bacteria species, or serogroup of species, in CFU/ml, in the water being tested.

26 The method of claim 25 in which the *Legionella* bacteria species, or serogroup of a species, in step (a) is a serogroup of *Legionella pneumophila* bacteria and the polyclonal [raw] antibodies of step (d) are polyclonal [raw] antibodies to bacteria of the same serogroup of *Legionella pneumophila.*

27 The method of claim 26 [25] in which the bacteria of a serogroup of *Legionella pneumophila* are bacteria from serogroup 1.

30 A method according to claim 25 [27] wherein the enzyme immunoassay is run as a sandwich assay.

31 A method according to claim 25 [28] wherein the enzyme immunoassay is run as a competitive assay.

32 A method according to claim 25 wherein the coated solid substrate in step (f) (i) is the inner wall of a test tube, which test tube also serves as the test reaction vessel in [step (e)] steps f (iii) and f (iv).

33 A method according to claim 25 wherein the coated solid substrate in [of] step (f) (i) is selected from among coated solid inserts and coated beads.

34 A method according to claim 25 wherein the enzyme of the conjugate prepared [reacted] in step (f) (ii) is horseradish peroxidase and a colorimetric agent, tetramethylbenzidine, is added in step (f) (iv).

35 A method according to claim 25 in which the buffer solution of step f (iii) is aqueous 0.05 [0.5] M tris [H Cl] HCl containing 2-5% of a detergent having a pH of about 7.0.